APPENDIX 3.X.X.

GUIDELINES FOR SOMATIC CELL NUCLEAR TRANSFER IN PRODUCTION LIVESTOCK AND HORSES

PREFACE

Following the first meeting of the OIE *ad hoc* Group on Biotechnology held from 3 to 5 April 2006, the Biological Standards Commission suggested restricting the mandate "to develop guidelines on the animal health risks arising from somatic cell nuclear transfer (SCNT) cloning of production animals, including criteria for assessing the health of embryos and animals derived from such cloning." The following document is a starting point for identifying, characterising and providing a basis for discussion on the animal health risks associated with SCNT cloning technology.

Overview

At the first meeting of the *ad hoc* Group on Biotechnology, it was recommended that the Subgroup on Reproductive Animal Biotechnologies should draft guidelines on risk analysis, based on the life-cycle approach, for biotechnology-derived animals. The definition of 'Reproductive Animal Biotechnology' was proposed as "the generation of animals through the use of assisted reproductive technologies (ART), which range from artificial insemination through to technologies involving a significant *in-vitro* component, such as *in-vitro* fertilisation, embryo transfer, embryo splitting and including asexual reproduction such as nuclear transfer". The following ~~draft~~ text is restricted to SCNT and is based on a risk analysis approach to biotechnology-derived animals categorised according to the life-cycle approach consisting of: i) embryos, ii) recipients, iii) offspring, and iv) progeny of animal clones.

Scope

These guidelines address animal health aspects of production animals derived from some reproductive biotechnologies.

Recognising the mandate of the OIE and the suggestion of the Biological Standards Commission, it is the recommendation of the *ad hoc* Group on Biotechnology to identify risk analysis parameters for animal health and their implication for environmental safety and food and feed safety. These guidelines will focus initially on the scientific basis for the risk assessment aspects, prevention measures and guidance for production livestock and horses derived from ~~ART~~ SCNT cloning. This is without prejudice to the addition of any relevant issue at a later stage. At present, these guidelines include the following:

- Identification of animal health risks and recommendations for management of those risks in embryos, recipients, animal clones and progeny of clones;
- Risk and prevention measures related with SCNT cloning technology;
- Some welfare issues related to animal health.

Recognising further that the following issues have been discussed or may be addressed by other bodies or instruments, or that they may be addressed at a later stage by the OIE, the document does not address:

- Safety and nutritional aspects of food derived from ART, for example transgenics (addressed by Codex);
- Risks related to the environmental release of animal clones;
- Risks related to transgenic animals that have not involved SCNT or other cloning technology;
- Non-reproductive animal biotechnologies;
- Risks related to animals produced for xenotransplantation or organ donors;
- Technologies related to stem cells;
- Risk related to aquatic animal health, including fish clones;
- Risks related to other terrestrial animals, such as wild mammals and non-mammals, including avian species and insects

Background

Risk analysis– general principles

Risk analysis in general includes hazard identification, risk assessment, risk management and risk communication. The risk assessment is the component of the analysis that estimates the risks associated with a *hazard* (~~OIE *Terrestrial Animal Health Code* [*Terrestrial Code*], 2006,~~ see Chapter 1.3.1.). These principles are routinely used by regulators in making decisions about experimental or commercial releases. These analyses can then be used to determine whether the outcomes require management or regulation. Risk management is the process by which risk managers evaluate alternative actions or policies in response to the result(s) of the risk assessment taking into consideration the various social, economic, and legal considerations that form the environment in which such activities occur.

For animal diseases, particularly those listed in the ~~OIE~~ *Terrestrial Code*, there is broad agreement concerning the likely risks and these risks can be qualitative or quantitative (~~OIE *Terrestrial Code*,~~ see Chapter 1.3.1). In disease scenarios it is more likely that a qualitative risk assessment is all that is required. Qualitative assessments do not require mathematical modelling to carry out routine decision-making. Quantitative or semi-quantitative risk assessments assign magnitudes to the risks in numerical (e.g. 1/1,000,000) or ~~verbal~~ descriptive (high/medium/low) terms.

In the context of animal cloning, two broad categories of risk assessments are considered: absolute risk assessment and comparative risk assessments. Absolute risk assessments characterise risk independent of a comparator (e.g. the likelihood of an animal transmitting a specific livestock disease). A comparative risk assessment (or relative risk assessment) puts the risk in the context of a comparator. For example the degree to which an animal produced by one reproductive technology can transmit a particular disease to another animal of the same species compared with the degree to

which a similar animal produced by another reproductive technology transmits the same disease to another animal of same species.

Regardless of the methodology used, hazard identification is an early step in all science-based risk assessments. In the context of assessing the risks associated with animal cloning (SCNT) and starting with the embryo and moving on through animal clone development and subsequent progeny, it is important to be clear at this juncture that only a comparative semi-quantitative risk assessment can be completed. A systematic, absolute, quantitative risk assessment of potential risks is difficult, due to the relative newness of the technology, and the variability in outcomes among laboratories and species cloned. Furthermore, with the technology of SCNT there is no introduced hazard from the insertion of novel genes (which may potentially happen in transgenesis). Thus, to analyse what factors contribute to animal health risks, the existing baseline must be analysed.

In short, the specific points where the risk assessment needs to be focused need to be identified. As illustrated in the accompanying diagram – the focus is to look at the basics of creating an embryo – using current terminology, starting from the selection of donor of oocyte and the cells to the creation of an embryo by the cloning methodology. The second phase will focus on the recipient of the embryo clone and the animal health and care considerations for the animals. The actual embryo clone that is born as an offspring is the third part of the paradigm that needs clear guidelines for assessment, and the next generation, either the progeny of the animal clone (which is a result of normal sexual reproduction) or animals produced by recloning (clones of clones) is the fourth and final stage.

Managing Animal Health Risks associated with embryos

Embryo production by *in-vitro* techniques has been applied for many years. Although the additional steps involved in cloning add a new dimension to this procedure, many of the risks associated with SCNT have previously been identified for established ART (~~OIE, *Terrestrial Code*,~~ see Appendix 3.3.2.). An analysis of SCNT methodology allows the procedural details to be categorised into:

i) Oocytes (obtained from the abattoir, recovered from trans-vaginal ultrasound-guided procedures or by laparotomy procedures).

   The primary risks are associated with the health status of the animal from which the ovaries are harvested and the quality of the oocytes.

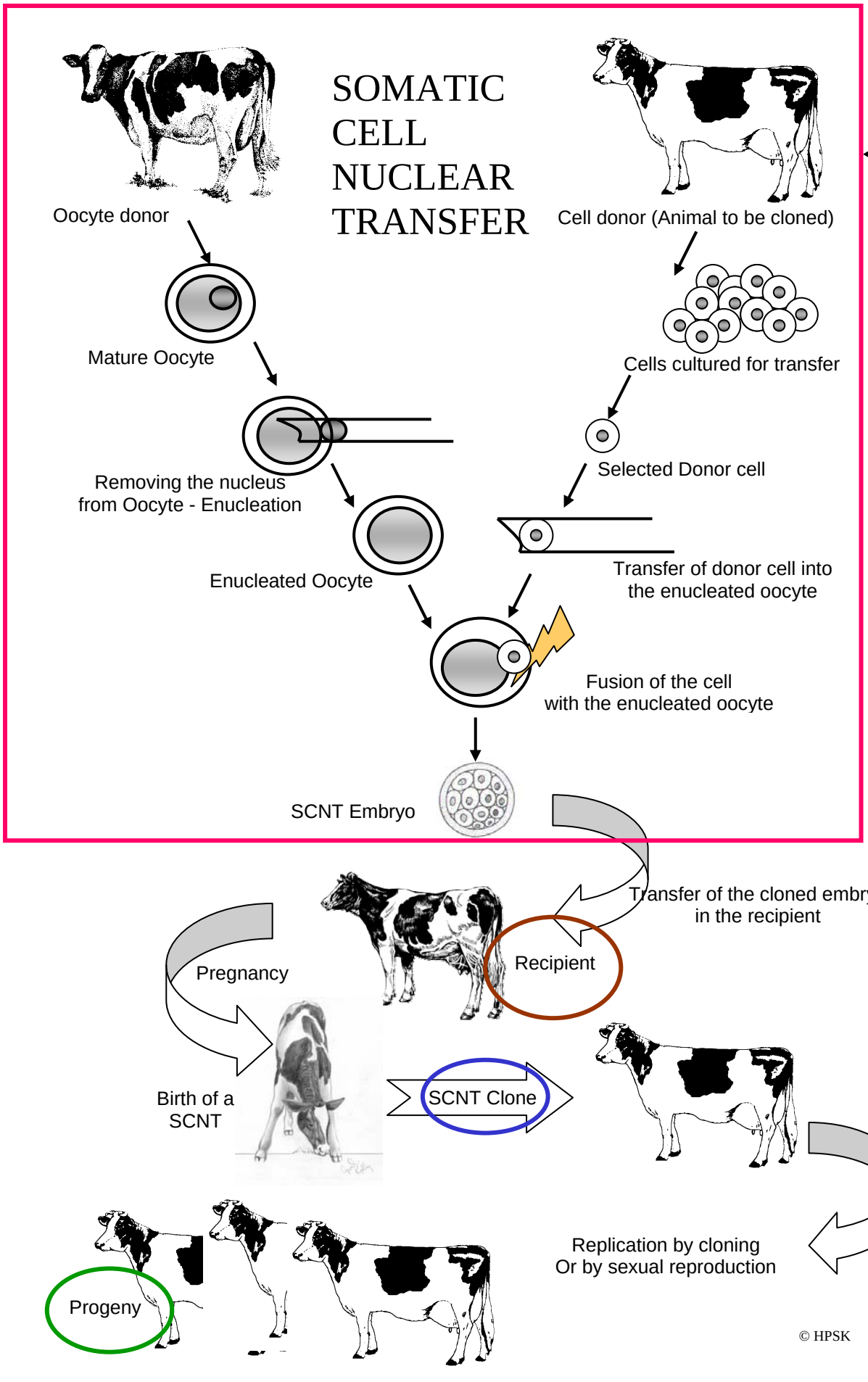
ii) Donor cells (cells obtained from animals chosen to be cloned – by biopsy, harvesting at slaughter or after death).

   Currently there are no specific new risks identified with SCNT cloning. There is a proposed risk related to activation of endogenous retroviruses during cell transfer procedures, however, this may be more theoretical than practical. In some current experimental procedures, the donor cell may be treated with chemicals to modify its composition, for example cell cycle inhibitors or chromatin modifiers.

iii) *In-vitro* culture of reconstructed embryos (procedure used to fuse the donor and recipient material and to culture the reconstructed embryo).

   Risks associated with the method of fusing donor cells with enucleated recipient oocytes and with culture conditions.

In addition, the practitioner should ensure that the clone pregnancy is compatible to the surrogate dam's breed, anatomy and physiology.


SOMATIC CELL NUCLEAR TRANSFER
Oocyte donor
Cell donor (Animal to be cloned)
Mature Oocyte
Cells cultured for transfer
Selected Donor cell
Removing the nucleus from Oocyte - Enucleation
Enucleated Oocyte
Transfer of donor cell into the enucleated oocyte
Fusion of the cell with the enucleated oocyte
SCNT Embryo
Transfer of the cloned embryo in the recipient
Recipient
Pregnancy
Birth of a SCNT
SCNT Clone
Replication by cloning Or by sexual reproduction
Progeny

Oocytes

- The laboratory or the producer should establish a detailed record of ovaries – their origin, health of the animal from which the ovaries are obtained, details of any systemic lesion on the animal and proper herd data. This is particularly useful where the pooling of ovaries may provide cross-contamination of ovarian tissue.

- Follicular fluids may carry various infectious agents like bovine viral diarrhoea virus (BVDV) and can contaminate pooled follicular fluid from healthy animals. Furthermore, the technique for collecting oocytes, such as aspiration or slicing of the ovarian follicles, determines the extent of blood contamination or extraneous material. A representative sample to demonstrate the absence of infectious biological material should be done with each pooled batch.

- Oocytes are matured as cumulus oocyte complexes (COCs) and then matured in most instances in the culture/maturation media. Care and efforts should be taken to carefully select and mature the oocytes from the pools that are morphologically good; also the media used should have been quality tested. Use of serum or protein components from an undefined or untested source should be avoided. Addition of proper and safe antibiotics in the culture media to control opportunistic bacteria should be encouraged.

- Use of proper sanitary and disinfection procedures is of utmost importance and should be emphasised in any *in-vitro* fertilisation (IVF) laboratory. Proper handling and following sanitary protocols during the maturation and further culture of embryos should be encouraged.

Donor cells

In order to minimise risks

- Donor cells should be properly harvested from the animal and cultured under proper sanitary conditions using good laboratory practices.

- When applicable, the passaging of the cells used for the cloning procedure should be documented and at different stage sampling may be warranted to look at the chromosomal component of the cell lines. If possible, procedures should be in place for regular sampling of the cells for morphological and other characteristics.

- Master cell lines (to be used for cloning at a later stage) should be stored under conditions found to be optimal for maintaining viability. Freedom from extraneous agents should be established by testing for bacteria, fungi, mycoplasmas or viruses, using appropriate tests (IETS Manual~~, 1998~~). –

Cloning procedures/reconstruction

- The cloning procedure that employs the use of chemicals or other reagents should be carefully evaluated, in terms of the quality of embryos and overall efficiency.

- During the fusion of recipient and donor material by chemical or physical means care and control should be employed. The optimisation of the procedure based on the laboratory protocols or published reports should be determined to avoid early embryonic mortalities.

- If co-culture of the cell is used for the culture procedure after reconstruction of embryos, proper screening of the co-culture cells should be done. A sample of each batch may be tested for the bacterial, fungal, mycoplasmal or viral component.

- Embryos should be cultured and harvested for an appropriate time and stage to transfer them or to cryo-preserve them for later use. Proper procedures based on the international standards (IETS Codes of Practice) for washing and preservation of the embryos should be followed.

- Care should be taken with regard to grading the embryos before transfer (~~OIE *Terrestrial Code*~~, Appendices 3.3.1 and 3.3.2).

Managing animal health risks related to the recipients (surrogate dams)

1. Animal health risks to the surrogate dams

Currently, when compared with *in-vitro* produced embryos, SCNT has a higher rate of pregnancy failure and, in some species, placental abnormalities. Loss due to defects in the embryo or failure to implant in the uterus of the surrogate dam does not pose a hazard to the dam. Rather, the surrogate dam simply resorbs any embryonic tissue and returns to cycling. Mid- and late-term spontaneous abortions may be hazardous to surrogates if they are unable to expel the fetus and its associated membranes. Most abortions in natural service and artificial insemination (AI) pregnancies in cattle remain undiagnosed due to the expense of laboratory work and the low profit margin in both the beef and dairy industry. Producers and veterinarians become concerned when the rate of abortion exceeds 3–5% in a herd. The same potential impact of external influences should be considered with pregnancy evaluation with SCNT and other reproductive technologies. Disease, under-nutrition, and severe environmental conditions are stressors known to interfere with animal fertility and embryo survival. Under these circumstances, the risk to the pregnancy is directly related to stress factors and not to the technology used.

To date, a species-specific effect has been seen. Abnormalities in clones may result from incomplete reprogramming of the donor nucleus. Epigenetic reprogramming occurs at different times in embryos in different species. Many of the abnormalities reported in cattle and sheep pregnancies have not been noted in goats or swine carrying SCNT clones. The amount of *in-vitro* manipulation of an embryo inversely correlates to the chances for successful pregnancy outcomes. This has been observed in both SCNT embryos and *in-vitro* produced fertilised embryos. Unlike other forms of other reproductive technologies SCNT pregnancy losses occur at all stages of gestation in cattle. Clone pregnancies have been lost during the second and third trimesters and have been accompanied by reports of hydrops, enlarged umbilicus, and abnormal placentation.

2. Animal health risks posed by the surrogate dam to the clone embryos

No new animal health risks have been identified for the developing clone fetus from the surrogate dam compared with conventional pregnancies. The latter include vertically transmitted diseases and abnormalities due to metabolic or physiological stress.

With respect to the animal health risks associated with the surrogate dam, it is difficult to document the relative frequency of early stage losses of SCNT embryos compared with early

stage losses of other pregnancies as these abortions are not typically diagnosed with other reproductive technologies. Additionally, external stressors will similarly impact SCNT pregnancies.

Veterinarians should monitor the progress of pregnancy as the common gestational anomalies seen in other assisted reproductive technologies may be exhibited and diagnosed during the physical examination. A database of commonly encountered problems in clone pregnancies would be useful if available to animal health experts.

- Care should be taken to assess the general health of the recipient dam before selection to carry the embryo clones. The general health status of the recipient should be determined in terms of freedom from infection and disease, proper vaccination and follow up, and, if applicable, proof of earlier uneventful pregnancies, absence of birthing problems, and proper post-pregnancy recovery.

- Pregnancy loss is greatest with SCNT embryos prior to 60 days' gestation in cattle. This is similar to the pattern seen with other reproductive technologies. However, in clones, high pregnancy losses during this time of placental formation (between 45–60 days) suggest that embryonic death may be a consequence of faulty placentation. Abnormal placentation may lead to a build up of wastes in the fetus and associated membranes, or inadequate transfer of nutrients and oxygen from the dam to the fetus. Care should be taken to monitor the recipient dam during pregnancy. Once the pregnancy is established and confirmed, regular veterinary assessments and monitoring of animal health status is desirable up to the birth of the offspring.

- To ensure that the recipient is pregnant and to monitor its health during the first trimester, it is useful to perform ultrasonographic assessments, determine hormonal profiles and assess the general physiological parameters. Based on these profiles, proper attention should be paid to aid in the proper establishment of pregnancy by providing proper husbandry conditions and nutrition.

- The animals should be observed carefully for the signs of labour nearing the time of birth. In some species, one of the more common problems is uterine inertia and the absence of contractions. The absence of contractions may result in prolonged pregnancies with associated sequellae that may require assistance with deliveries.

- A surgical intervention should be decided and should be available for the near term animal if the situation so warrants. Proper procedures should be employed to ascertain the proper handling of the offspring and the surrogate dam.

- Health concerns may arise as a result of surgical procedures, excessive traction, or other complications such as retained fetal membranes. In these cases *post-partum* care may be necessary.

Managing animal health risks of animal clones

The health problems of individual clones can be observed *in utero* and *post-partum*. These appear to be the same as observed in other ART, but they may be more common in clones. It is important to determine whether the abnormalities are of genetic or epigenetic origin. Large offspring syndrome (LOS) and placental abnormalities are particularly observed in sheep and cattle.

- Appropriate husbandry practices are important to the health of animal clones. Care should be taken to provide colostrums and a clean and hygienic environment, supervision for the first few weeks after birth should be practiced.

- The animal clones must be checked routinely for the most common phenotypic anomalies, such as atresia anii, umblical hernia, flexor muscle contractions, respiratory or cardiac insufficiency, and failure to suckle. This will allow proper treatment and care of the newborn and increase the survival of the young one.

- To consolidate current understanding of the health status of animal clones, a comprehensive veterinary examination should be performed to monitor the progress of the clone, as unexplained fatalities or fatalities arising from systemic complications have been reported. It is encouraged to follow the health profile of the animals to at least the reproductive maturity stage, and to record the ability to reproduce (fertility index).

- Animal welfare concerns ranging from LOS to serious abnormalities are notable in the debates pertaining to cloning technology. Proper research and peer-reviewed data should be generated. The animal clones should undergo species-specific basic welfare assessments. If welfare concerns are detected at initial screening, a more extensive characterisation of that phenotype should be performed to document the animal welfare concerns.

- Proper monitoring of the animal population during different stages of life from birth to puberty should be documented to address and validate the genomic potential of the animal clones.

Managing animal health risks related to sexually reproduced progeny of clones

Presently there is no evidence of an increased health risk if sexual reproduction is used for obtaining progeny. Some data indicate that the reprogramming errors during the cloning process may actually be corrected during the natural mating and reproduction process.

- Characterisation of the health profile, including health status and data on animal welfare, would consolidate the knowledge of sexually reproduced progeny.

- Monitoring the reproductive performance of sexually reproduced progeny of clones would be useful to assess their reproductive capacity in comparison with their conventional counterparts.

Managing animal health risks associated with re-cloning/clones of clones

Information on recloning is only beginning to appear. It is therefore necessary to follow the approach below:

- The health profile (health status and data on animal welfare) should be characterised to consolidate the knowledge.

- The reproductive performance of clones of clones should be monitored to assess the capacity of the animals to perform in comparison with their conventional counterparts.

Review of guidelines

The goal of these guidelines is to provide a scientific basis and recommendations on animal health and welfare risks to animals involved in SCNT cloning compared with other ART. These guidelines will focus initially on the scientific basis for the risk assessment aspects, prevention measures and

guidance for production livestock and horses, derived from ~~ART~~ SCNT cloning and should be reviewed in light of new scientific information.

~~***Glossary:***~~

~~**Hazard: (as defined in OIE)**~~

~~Hazard means a biological, chemical or physical agent, or a condition of, an animal or animal product with the potential to cause an adverse health effect.~~

~~A hazard is an element or event that poses potential harm; an adverse event or adverse outcome. A hazard is identified by describing what might go wrong and how that might happen. Covello and Merkhofer defined a hazard as a (potential) source of risk that does not necessarily produce risk. A hazard produces risk only if an exposure pathway exists and if exposures create that possibility of adverse consequences. Hazard identification is the process of identifying new agents in sources of risk. Risk sources may release risk agents into the environment.~~

~~**Risk:**~~

~~Risk means the likelihood of the occurrence and likely the magnitude of consequences of an adverse event to animal or human health during a specified time period, as a result of hazard.~~

~~The likelihood of the occurrence and the magnitude of the consequences of an adverse event; a measure of the probability of harm and the severity of impact of a hazard. Objective measurement and scientific repeatability are hallmarks of risk. In risk studies it is common, especially in oral communication, to use "risk" synonymously with the likelihood (probability or frequency) of occurrence of a hazardous event. In such instances, the magnitude of the event is assumed to be significant.~~

~~**Risk analysis:**~~

~~Risk analysis means the process composed of hazard identification, risk assessment, risk management and risk communication.~~

~~The process of risk analysis includes risk assessment, risk management and risk communication.~~

~~**Risk Assessment:**~~

~~Risk assessment means the evaluation of the likelihood and biological and economic consequences of entry, establishment, or spread of a pathogenic agent.~~

~~The process of identifying a hazard and evaluating the risk of a specific hazard, either in absolute or relative terms. The risk assessment process involves four interrelated assessment steps: release assessment, exposure assessment, consequence assessment and risk estimation. It includes estimates of uncertainty in process, and is an objective, repeatable, scientific process. Quantitative risk assessment characterises the risk in numerical representations. Qualitative risk assessment characterises the outputs on the likelihood of the outcome or the magnitude of the consequences in qualitative terms such as "high", "medium", "low" or "negligible".~~